

# Cold tolerance of rice plants transformed with *Arabidopsis* H<sup>+</sup>/Ca<sup>2+</sup> antiporter *CAX1* gene

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## Objectives

This study was carried out to evaluate the cold tolerance for the rice plants expressed *CAX1* (accession no U57411) gene.

## Materials and Methods

- o. Plant materials: 7-day-old seedlings of T<sub>3</sub> lines
- o. Cold treatment condition : 14 days at 17°C.
- o Analysis of chlorophyll content · leaf of transgenic plants treated for 14 days at 17°C

## Results and Discussion

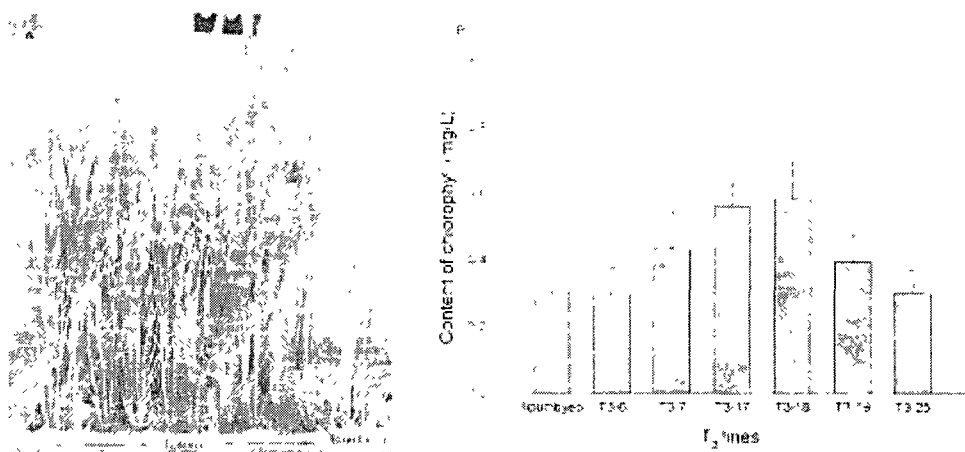
In the previous study, we successfully developed the transgenic rice plants overexpressing the *Arabidopsis* H<sup>+</sup>/Ca<sup>2+</sup> antiporter *CAX1* gene. The 7-day old seedlings of transformants were treated for 14 days in growth chamber controlled 17°C. Cold tolerance of transgenic plants were compared with the donor rice cultivar, 'Ilpumbyeo'. Most of transgenic plants appeared to have cold tolerance compared with wild types. The chlorophyll content of T<sub>3</sub> transformants was higher than that of wild type. The gene of *A. thaliana* H<sup>+</sup>/Ca<sup>2+</sup> transporters, *CAX1* provides resistance to abiotic stresses in most crops(Hirschi et al. 1996).



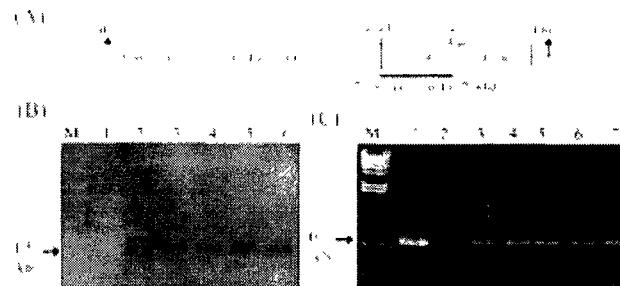
**Fig. 1** Development of calcium-rich rice. (A) induction of seed-derived callus on modified N6 medium, (B) green spot on co-cultivation of rice callus which *Agrobacterium* vector (pCAX1), (C) plant regeneration from callus, (D) ripening stage of the transgenic plants.

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**Fig. 2.** Cold tolerance of T<sub>3</sub> plants treated for 14 days at 17°C. A: Phenotype compared with wild type plant and transformants, B: Chlorophyll content compared with wild type plant and transformants.



**Fig. 3** DNA gel-blot analysis. (A) Schematic diagram of part of the T-DNA region of vector pCAX1. Nos-pro, promoter of nopaline synthase gene; NPT II, neomycin phosphotransferase II gene; Nos-ter, terminator of nopaline synthase gene; 35S pro, CaMV 35s promoter, (B) Southern blotting hybridization pattern of DNA prepared from T<sub>1</sub> transformed plants and an untransformed plant. Genomic DNA was digested with *Xba* I and *Sal* I fractionated on 0.8 % agarose gels, and transferred to nylon membranes. The membrane was hybridized with <sup>32</sup>P-labeled fragment of *CAX1* probed. M; λ*Hind* III digested marker, 1; control plant, 2~6, transgenic plants, (C) PCR analysis of genomic DNA from control and T<sub>0</sub> rice leaf tissues. PCR products amplified from putatively transformed tissues were visualized by electrophoresis on a 0.8 % agarose gel stained with ethidium bromide. M; λ*Hind* III digested marker, 1; pCAX1 vector, 2; untransformed plant, 3~7: transformed plants.

### References

Hirschi KD, Zhen RG, Cunningham KW, Rea PA, Fink GR. 1996. *CAX1*, an H<sup>+</sup>/Ca<sup>2+</sup> antiporter from *Arabidopsis*. *Proc Natl Acad Sci USA* 93:8782-8786.